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RESEARCH ARTICLE.........

Study of HACCP and microbial quality of seafood during processing in plants around Kolkata

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ABSTRACT...... Food quality has become a considerable issue in food sector over the past decade. The food safety concepts are gaining attention not only in developed countries but also in developing countries like India. Finfish and shellfish are some of the highly perishable commodities, so, systematic approach is required to maintain quality during processing. There is a worldwide trend towards food safety and quality in order to prevent food borne illness. For ensuring food safety there are several quality control systems, out of which the Hazard Analysis and Critical Control Point (HACCP) is the most acceptable. In the present study, a survey was made to put an idea of implementation Quality Assurance and HACCP system in seafood plants in West Bengal. The bacteriological loads in all these plants were studied and found acceptable as per ICMSF (1988) guidelines. Further, an attempt was made to identify Critical Control Point (CCP) during receiving end of raw material at seafood processing plants. Thrust was given to adopt Total Quality Management practices during inspection of processing plants.

KEY WORDS...... HACCP, CCP, Quality assurance, Bacterial load, Seafood

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INTRODUCTION..

Fish and shellfish contribute about 15.6 per cent of animal protein and 5.6 per cent of total protein supply worldwide. It is estimated that 60 per cent of world population meets their animal protein demand from fish. Protein, lipids and bioactive compounds from seafood's have unique features that make them nutritionally superior and healthier over their terrestrial counterparts (Gopal, 2010). Finfish and shellfish are some of the highly perishable commodities, so, systematic approach is required to maintain quality during processing. There is a worldwide trend towards food safety and quality in order to prevent food borne illness.

About 76 million cases of food-borne diseases, resulting in 325,000 hospitalizations and 5000 deaths, are estimated to occur each year in the United States of America (USA) alone (Mead *et al*., 1999). Five hundred and seventy-one outbreaks involving 2991 cases were due to finfish, such as tuna and grouper, 135 outbreaks (3156 cases) were due to molluscan shellfish, 129 outbreaks (2400 cases) were linkedto crab cakes and sushi, and 64 outbreaks (765 cases) were due to other seafood such as shrimp and lobster (De Waal *et al*., 2006). There are over 200 known microbial, chemical or

physical agents that can cause illness when ingested (Acheson, 1999). Over the last 20 years, at least in the industrialised world, food-borne diseases caused by bacteria, parasites, viruses and prions have significantly moved up the political agenda and generated, on occasions, substantial media attention. In an attempt to reduce disease burden, the monitoring of food-borne diseases and pathogens in the food chainhas been implemented and a farm-to-fork approach has been adopted encouraging all sectors of the food production chain to improve hygiene and actively incorporate structured approaches to food safety, such as HACCP principles (Newell *et al*., 2010). For ensuring food safety there are several quality control systems, out of which the Hazard Analysis and Critical Control Point (HACCP) is the most acceptable. Microbiological analysis is also an essential tool for carrying out tests in accordance with the microbiological criteria established for each food type, as well as being essential for evaluating the actions of different management strategies based on the Hazard Analysis and Critical Control Points (HACCP) system (Jasson *et al*., 2010). These new detection methods are the necessary technologies that will substantially improve our food safety once integrated in the HACCP (Bhunia, 2008).The Hazard Analysis Critical Control Point (HACCP) system identifies, evaluates and controls hazards that are significant for food safety (Ababouch, 2014). Adoption of HACCP will not only produce quality foods but also will help in ensuring food safety as per the specification of ICMSF (Nanda and Sarkar, 2010). Consumption of fish and shellfish has been associated with a variety of health hazards in human, like infection with pathogenic bacteria,virus, helminthes, allergens etc. Seafood could be exposed to a wide range of hazards origination from water to the table. Some of these hazards are natural to seafood's environment like toxin, virus, bacteria and others are introduced by humans.The hazards can involve bacteria, viruses, parasites, natural toxins, and chemical contaminants like antibiotics, pesticides, heavy metals. Consumption of seafood creates human health hazard due to presence of allergen through allergic reaction such as stomach and intestinal disorder, cancerous growths to general degeneration of peripheral cellular tissues, gradual breakdown of the digestive and excretive systemsetc.

The prerequisite programmes for HACCP for fish and shellfish consist of scrutinizing the water quality, sanitation, packaging and labeling. The requirement for hygienic practices constitute the prerequisite programmes that are essential for any food operation prior to the implementation of the HACCP system (Karunasagar, 2014). Considering its importance, the US Food and Drug Administration (FDA) adopted HACCP in 1970 in order to ensure the safety and quality of food sold in US market. Codex Alimentarius, an agency of the United Nations, adopted HACCP as a food safety standard in 1991. The original concern for food safety which initially focused on microbiological contamination was later broadened to include chemical and physical hazards like antibiotics and drug residues, pesticides, heavy metals etc.

The risks to consumer health from sea-foods captured in unpolluted marine environment are low, provided these products are handled in line with principle of good manufacturing practice (Reilly *et al.,* 1997). The risks of food borne diseases are higher with aquaculture products from in land or coastal ecosystems due to greater potential of environmental contamination, compared to capture fisheries. Most of the food safety hazards associated with aquaculture produces can be controlled by good fish farm management practices and appropriate consumer education regarding such risks from eating raw or improperly cooked products that may contain parasites and pathogenic bacteria. The introduction of safety assurance system based on the HACCP principles in farm management may initially add to the cost of aquatic products but is always beneficial in long run. Placing unsafe food products in market can be detrimental to the reputation of the fish farming sector, in terms of product rejection and costs of food borne diseases in national economics.

India is the second largest producerof fish in the world contributing to about 5.43 per cent of global fish production. India is also a major producer of fish through aquaculture and ranks second in the world after China. The total fish production during 2011-12 is at 8.67 million tones with a contribution of 5.30 million tons from inland sector and 3.37 million tons from marine sector, respectively. Fishery being one of the promising sectors of agriculture and allied activities in India, a growth target rate of 6 per cent was fixed so as to achieve the overall growth rate of 4.1 per cent for Agriculture during the 11th Five-year Plan (Annual report 2012-13, Dept. Animal Husbandry and Dairying, Govt. of India). India is one of major producers and exports of seafood products. It also

meets the outgrowing demand of fast growing economy and human population. During the financial year 2011-12 export of marine products from India reached in the tune of 862021 tons amounting to Rs.16597 crores and USD$3508.45 million (MPEDA, 2012-13). Generally, here to satisfy consumer needs, seafood products are sold as whole or processed like head less and deveined shrimps/ prawns, fish fingers, seafood mix, fish cutlets, fish patties and shrimp pickles etc. Applications of HACCP to aquaculture and every sphere of seafood processing are both a relevant and crucial component for its globally traded product (Panigrahi *et al.*, 2010).

The objective of the study is to conduct and quality assurance survey in some fish processing plants in and around Kolkata in order to assess the microbiological quality of processed fish and fishery products with special emphasis to identify the critical control points (CCP) during processing.

RESEARCH METHODS....................................

An initial quality assurance survey of seafood products (processed, packed, frozen and bulk-packaged of black tiger prawn, *Penaeus monodon*) purchased from different processing plants and handlers in and around Kolkata, India was conducted which resulted in a close interaction with the owners of processing plants, processors/handlers. Based onthe packaging method, location, type and number of consuming population, four processing plants (A, B, C and D) were selected for evaluation of microbiological quality and hazard analysis. There are 10 numbers of European Union approved and 20 numbers of non-registered seafood plants in and around Kolkata. During the study, visual hygiene analysis and quality assurance survey of plants were conducted. Shrimp samples and swabs of processing accessories were taken to determine the bacterial load by standard microbiological techniques.

10 g of the samples were collected from raw (unprocessed) and after each step of the processing to packaging and the finished product for sales. Swabs of processing tables, conveyor belts, fish handling box and grading tables were also collected. Samples were also purchased from vendors for microbiological analysis. All samples collected brought to laboratory with in 2 h of collection for analysis in ice cover. Samples that could not be analyzed were stored at -4^{0}C in a freezer till the following day while seafood products were maintained at -20^{0}C till the following day. Besides, sterile swabs were used for swabbing. A hollow wooden square frame was constructed taking 5 cm in length and 5 cm in width in order to give internal swabbing area of 25 cm^2.

The sections of muscles with skin / exoskeleton from shrimps were cut and homogenized in to 10 % (w/v) Phosphate Buffer Solution (PBS pH-7.3-7.4). The homogenized tissue samples were serially diluted in PBS and plated on to Tryptic soy agar (DIFCO, Becton Dickson and Company, Sparks Md., USA) for Aerobic plate count (APC) of bacteria and the plates were incubated at 37^{0}C for 24 h. Similarly, sterile swabs were used for swabbing (25 cm^2 areas) on processing equipment like tables, conveyor belts, fish handling box etc. in order to enumerate bacterial load. The swab samples were put in the TSB broth and incubated at 37^{0}C for 24 h. After incubation 0.1 ml of culture was poured and spread with a glass rod on TSA plate and again incubated at 37^{0}C for 24 h.

The statistical analysis of bacterial loads of all samples of shellfish was performed using SAS 9.2 software at α =0.05 significance. The figures and graphs were generated using JMP 8.0.2 software during the study.

RESEARCH FINDINGS AND ANALYSIS...........

The visual inspection of processing equipment to maintain hygienic condition for seafood processing (Table 2) showed that most the plants possess moderate to ideal processing condition. Presence of total bacteria in swabbing in fish processing plants in Kolkata is given in Table 3. The total plate count was maximum in fish processing equipment sample (75 x 10^{2}cfu/25cm^2) and working table for grading (46 x 10^{2}cfu/25cm^2) collected from processing section. The minimum total count was observed in (11.6 x 10^{2}cfu/ 25cm^2) fish handling box No.1 from the receiving section.

The total bacterial load of shrimps in different seafood processing plants in Kolkata showed significant variation ($P< 0.05$) among different plants (Fig. 1). The total bacterial load of shrimps in different seafood processing plants in Kolkata showed in significant variation ($P> 0.05$) among samples of different processing stages (Fig. 2).In the study it was found that the Aerobic Plate Count (APC) was high in raw shrimp in most of the Plants and bacterial load decreased gradually in all successive stages (Fig. 3).The bacterial load of shrimp

in Plant D is significantly higher than the rest of the plants like A, B and C as the plants were denoted by A,B, C, D. This is possible due to implementation of HACCP system and SSOP system by the plants A to C except Plant D. The total bacterial loads were recorded 10^4cfu/g during different stages of processing of shrimps which was lower than the standard limits of 5 x 10^5cfu/g as set by International Commission on Microbiological Specifications for Foods Standards (ICMSF, 1988). The bacterial load in fresh tropical shrimps was reported in the range from 10^3 to 10^5 cfu/g by several authors in previous studies (Iyer and Joseph, 1995).

Table 1 : Survey of quality assurance system in four fish processing plants Kolkata

Item	Seafood plants in and around Kolkata			
	A[a]	B[a]	C[a]	D[b]
Preprocessing facility	++	++	++	-
General Construction	++	++	++	++
Air cushion	++	++[c]	+++	++
Air conditioning system	+++	+++	+++	++
Ice Plant	++	++	++	+
Chill room	++	++	++	++
Laboratory facilities	++	++	+++	+
Changing rooms	+	+	+	+
Wash rooms	++	++	+++	++
Water tanks	++	++	++	++
Water treatment plants	+	+	+	+
Processing tables	+	+	++	+
Cold storage	+++	+++	+++	+++
Processing equipments	++	++	+++	+
Packaging facilities	+	+	++	+
Effluent treatment plants	+	+	+	-
Thermographs	+	+	+	-
Cleaning and Sanitation facilities	++	++	++	+
Chlorinated water facilities with Chlorine doser	+	+	+	+
Medical checkup facilities for workers and staff	+	+	+	+
HACCP implementation	++	++	+++	+
Standard Sanitary Operation Procedure (SSOP) implementation	++	+++	+++	+
Good manufacturing practices(GMP) implementation	+++	+++	+++	+
Cold chain facilities	++	++	++	+

[a.] The EU, EIA approved plants
[b.] The Non-EU approved plant [c.] Present or absent of facility or quality system is indicated by + or – sign, respectively. The maintenance of quality is arbitrarily approximated by number of + sign.

Table 2 : The visual inspection of processing equipment to maintain hygienic condition for seafood processing

Areas	Equipments	Surface material	Visual Inspection			
			A	B	C	D
Receiving	Defrosting unit	Polyethylene	1[a]	1	1	2
Do	Grader	Stainless steel	2	1	1	1
Peeling	Peeling machine (roller)	Rubber with nylon	1	1	1	2
Spreading	Conveyor Belt	Polyethylene	1	2[b]	1	1
Freezing	Plate Freezer	Stainless steel	1	1	1	2
Do	IQF Machine	Stainless Steel	1	1	1	1

Evaluation of cleaning based on following two levels
[a]1: Ideal condition for processing *i.e.* cleaned carefully with no debris of seafood
[b]2: Moderate condition for processing *i.e.* cleaned with negligible quantity of debris of seafood.

In the study, it was found that the Total Plate Count (TPC) was high in raw shrimp in most of the Plants and bacterial load is decreasing gradually over time in all successive stages. It is evident that the microbial load decreases after washing in chlorinated water and freezing. Similar findings were described by Alam *et al.* (2010). However, the total bacterial load of shrimps in different seafood processing plants in Kolkata showed in significant variation ($p > 0.05$) among samples of different processing stages which needs further work to draw a significant conclusion.The total plate counts were maximum in fish processing equipment sample (75 x 10^2cfu/25cm^2) and followed by working table samples (46 x 10^2cfu/25cm^2) collected from processing section.

Table 3 : Presence of total bacteria in swabbing in fish processing plants in Kolkata

Place of sampling	Swabbing objects	Total bacterial load (x10^2) (cfu/25 cm^2)
Receiving section	Fish receiving table	32
	Fish handling box	11.6
Processing section	Working table for grading	46
	Conveyor belt	39
	Processing equipments	75
	Fish handling box	26

Table 4A : Critical control point at receiving end of raw materials in seafood plants

CCP	Hazard	Critical limit	Monitoring				Corrective action	Record	Verification
			What	How	Frequency	Who			
Raw material	Antibiotic	Absent	Presence of antibiotic	Declaration from the supplier for non usage of antibiotic and Antibiotic testing of material in lab	Testing once in two months to cover all supplier	Qual ity assur ance team	Rejects lot if not accompanied by certificate and stop all purchase from supplier if tests are positive	Supplier 's declaration form, external test reports for antibiotic in two months in two months interval	Verification once in a week Review the external lab test report done once in two months
	Pesticide	Absent	Presence of pesticide	Declaration from the supplier for non usage of pesticide and pesticide testing of material in lab	Do	Do	Do	Do	Do

[a.]CCP : Critical control point is an important step of HACCP.

Table 4B : Critical control point at receiving end of raw materials in seafood plants

CCP	Hazard	Critical limit	Monitoring				Corrective action	Record	Verification
			What	How	Frequency	Who			
Raw material	Sulphite	Absent	Presence of sulphite	Malachite green test	Testing after each purchase	Quality assurance team	Stop all purchase from supplier if tests are positive	Supplier 's declaration form, test reports for sulphite	Verification once in a week Review the lab test report done once in two months
	Pathogenic bacteria	Absent All the baskets must bear a tag that discloses the date, time and place. Supplier's certificate that shrimps are handled and transported in hygienic manner.	Basket tag and supplier certificate	Visual/ analytical	Each basket and each lot	Quality control staff and receive in charge	Reject without tagging basket and supplier's certificate	Receiving tag and copy of Supplier's certificate Corrective action records	Daily receive record review Microbiological test of raw shrimp and end product

[a.]CCP : Critical control point is an important step of HACCP

The minimum total count was observed in (11.6 x 10^2cfu/ 25cm^2) fish handling box number 1 from the receiving section. The maximum count of TPC in processing equipment is due to the removal ofintestine from the fish leads to the release of gut flora in the cutting board of processing machine and cross contamination by handling. Similar results were observed by Prabakaran *et al.* (2011). During cross contamination, the bacterial transfer rates varied in high magnitude depending on the nature of surface (Chen *et al.*, 2001). Mandal *et al.*(2015) indicated that maximum microbial load in cross contamination condition occurred owing to transfer of equipment and products from one place to another.

Survey of quality assurance systemin four fish processing plants Kolkata is presented in Table 3. The result showed Plant A, B, C had more successfully adopted and implemented HACCP quality assurance system than plant D. The Critical Control Point (CCP) at receiving end of raw materials in seafood plants is

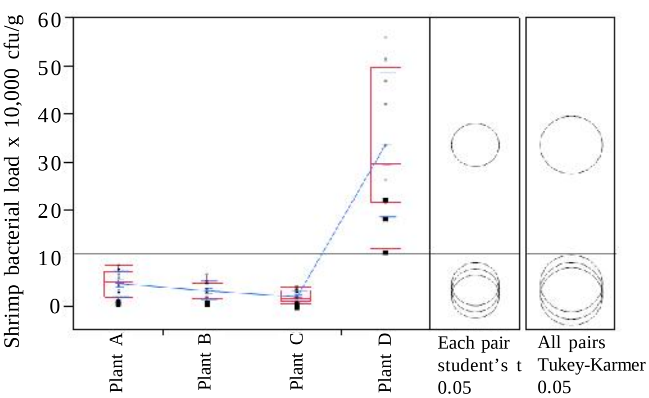

Fig. 1 : The total bacterial load of shrimps in different seafood processing plants in Kolkata showed significant variation (P< 0.05) among different plants

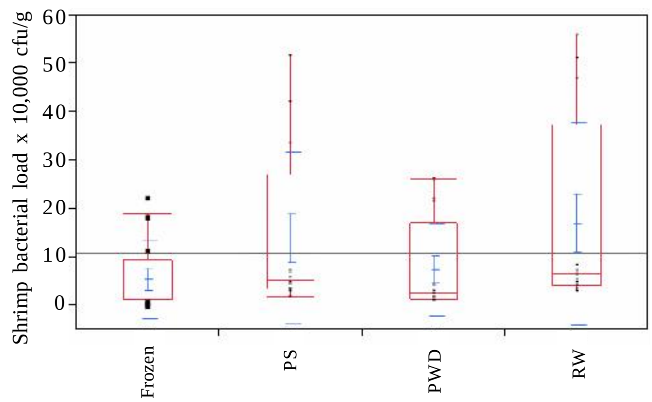

Fig. 2 : The total bacterial load of shrimps in different seafood processing plants in Kolkata showed in significant variation (P> 0.05) among samples of different processing stages. RW – raw shrimp, PWD- Peeled and Deveined shrimp, PS- Peeled shrimp, Frozen- Frozen shrimp

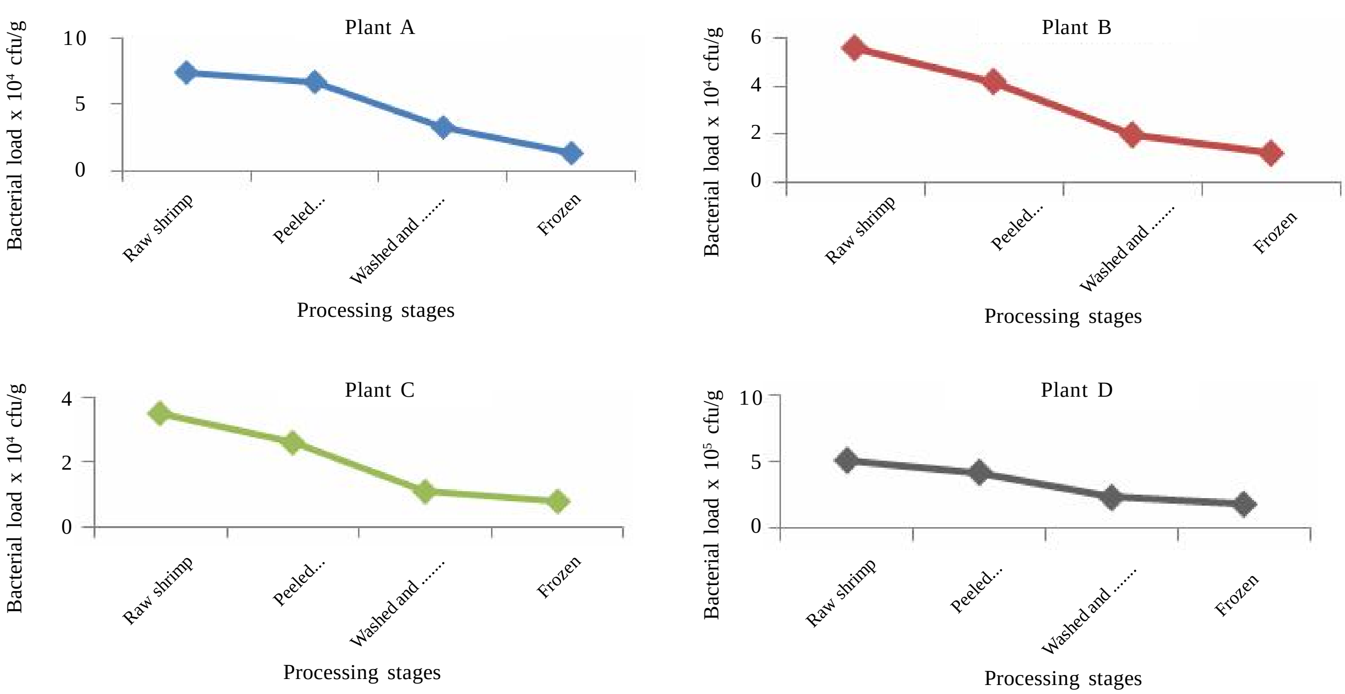

Fig. 3 : Bacterial load in different stages of shrimp processing in fish processing plant A, B, C, D in Kolkata

depicted in Table 1 and 4. Similar findings were also observed by Mandal *et al.* (2015). The present finding indicates that the adoption of Good Manufacturing Practice (GMP) and HACCP substantially improves the quality of the finished products.Moreover, the sanitary conditions of the fish processing plants are closely associated with the microbial quality of the finished product (Duran *et al.*, 1983).

The critical control point (CCP) of raw material in seafood processing plant is very important in quality control arena. In the present study, an attempt is made to trace out the specific CCP point to hold the quality of seafood. The source of pathogenic bacteria may come from natural sources and unhygienic handing of shrimp by the workers. Natural source is the culture farm from where good aquaculture practice is not properly maintained. Culture farm is the main source of bacteria. After harvesting, the time gap before reaching the processing plant decreases the quality of shrimp due to rapid growth of spoilage bacteria. In the processing plant, there is a provision for hand dip in 50 ppm chlorinated water and foot dip in 200 ppm chlorinated water for works as an instance of good hygienic practice (GHP). If the workers do not wash their hand and foot properly then the pathogenic bacteria may contaminate the shrimp during handing. The HACCP should be maintained in seafood processing plants in order to sustain the quality of shrimp.

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